SYMPOSIUM ON ACUTE ANTERIOR POLIOMYELITIS

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POLIOMYELITIS VACCINE:

*Some Considerations Relating to Its Safety**

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I AM grateful for this opportunity to come before you and discuss a subject of mutual interest—the production and safety of poliomyelitis vaccine. Certainly this matter is of particular interest to pediatricians, who will administer the bulk of the vaccine, as it is to us of the Public Health Service who must attest to its "purity, potency, and safety" under the Biologics Act of 1902. I will review some of the technical problems of vaccine production and testing as these developed before the general licensing of the product and shortly thereafter, when an outbreak of poliomyelitis occurred among children who had received vaccine distributed by one of the manufacturers. I will also discuss certain more recent developments that have resulted from a searching inquiry conducted this summer and fall by the Technical Committee on Poliomyelitis Vaccine.†

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Background: The vaccine as prepared for the field trial in 1954 was an experimental product made for the National Foundation of Infantile Paralysis by commercial producers according to the specifications of Dr. Jonas Salk and associates.[1,2] After some initial difficulty, the industrial firms were able to turn out a suitable product, and the field trial was carried out successfully. The results of the field trial were announced on April 12, 1955.[3] On that date, the Public Health Service with the advice of a panel of senior consultants issued official Minimum Requirements,[4] and six manufacturers that had produced vaccine under the earlier provisional requirements were granted licenses.

On April 26, six cases of poliomyelitis were reported among children who had received vaccine manufactured by the Cutter Laboratories. The ensuing investigation of the Cutter Laboratories was later extended to the entire industry and led to a temporary suspension of the nationwide vaccination program. Meanwhile the Public Health Service, on April 28, established a Poliomyelitis Surveillance Unit within its Communicable Disease Center in Atlanta, Georgia. This unit with collaborating state authorities investigated all reported cases of poliomyelitis, more particularly those associated with vaccine. The epidemiological data so developed clearly defined the Cutter incident as an outbreak with characteristics of a common-source epidemic.

This was the take-off point for a study of the vaccine—primarily the premises underlying the process and the extent to which it was amenable to application in routine large-scale manufacturing. The study can best be understood if I take a few minutes to review the vaccine's characteristics and their effect upon its production in the fall and winter of 1954-55.

Basic Procedures: In the production of the Salk vaccine, the basic procedures are the tissue culturing of three types of virus and their inactivation without excessive loss of potency. One strain of poliomyelitis virus has been selected for each type of poliomyelitis vaccine included in the final product. The current Salk formula recommends the Mahoney strain for Type 1, the MEF strain for Type 2, and the Saukett strain for Type 3. In the selection of these strains, the primary characteristic considered was their antigenicity.

Poliomyelitis virus of each type is propagated in a mass-production procedure by seeding minced or trypsinized monkey kidney tissue suspended in nutrient media with live virus. Shed virus is harvested by

simple filtration.

The procedure recommended for production involves inactivation of each single-strain suspension in 1:4000 formaldehyde, at 37° C at pH 7.0, for a period of time that provides for anticipated destruction of infectivity.

The process of inactivation was believed to follow the course of a first-order reaction yielding a straight line when the log of the viral concentration is plotted against time.[1,2] The course of the reaction is measurable with moderate precision during its initial phase, and it is postulated that a continuation of the reaction could be described by extrapolation of the straight line into ranges of viral concentration that are not measurable (Figure 1).

It was initially recommended that the inactivation treatment should be continued over a period equal to three times the period from zero time to the curve intercept at zero. Negative tissue-culture tests were anticipated during the latter portion of this period.

In producing the final vaccine, equal volumes of single-strain pools from each type of poliomyelitis virus are mixed. This mixture is termed a polyvalent or trivalent pool. Trivalent pools were judged suitable for manufacture of vaccine if they passed a second safety test consisting of monkey and tissue-culture tests in which at least 0.1 per cent of the total trivalent-pool volume was used as a sample. Testing is also directed toward the exclusion of B virus, bacterial contamination, and other considerations.

Analysis of Basic Procedures

Inactivation: An important feature of the Salk conception of a suitable vaccine lies in the principle which presumes a large margin of safety built into the inactivation process itself. Figure 1 shows the theoretical course of an inactivation process starting with a concentration of tissue-culture infective doses of 1.0×10^6, a common concentration. During the initial phase of the inactivation process, the log of the virus falls linearly with time, reaching zero some time between 48 and 72 hours. The concentration of live virus is then about 1.0 tissue-culture infective unit per ml. of viral suspension. The chart has been drawn to show the intercept time of the inactivation curve and log zero at three days.

Extrapolating the course of the reaction as a straight line into areas

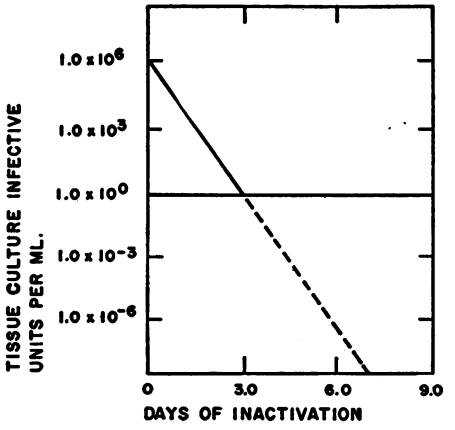

Fig. 1

of low viral concentration, the concentration is of such a low order that its significance can be grasped only when the numbers are reduced to common terms. If the reaction proceeds as initially postulated, there should be a concentration of live virus in the order of one tissue-culture infective unit in each million tons of inactivated viral suspension at the end of nine days.

There are alternatives, however, to this theoretical conception. A viral suspension is not in fact a monophasic system. It consists of a number of discrete particles in suspension. At high concentrations of virus, such a system may behave in a manner not dissimilar to a monophasic system. Thus, during the first part of chemical inactivation, the process may appear to behave as a simple first-order reaction. But as the reaction progresses and at lower virus concentrations, the heterogeneity of the system could well begin to assert itself and the initial description of the reaction no longer obtain.

This residual live virus will probably consist of particulates of varying size and shape and in varying degrees of aggregation. Large particulates, should they be contained in such a mixture, cannot be expected to inactivate at the rate calculated from the behavior of the reaction during its initial phase. During this initial phase, the rate of the reaction will be dominated by that portion of the particulates which compose the bulk and which are most easily inactivated. The extent to which the inactivation process departs from a straight line even in the initial phase of the reaction will be a reflection of the extent to

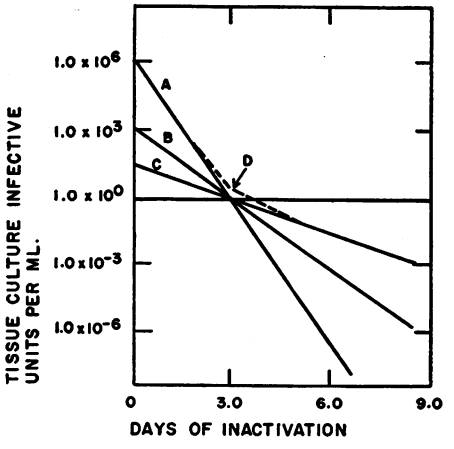

Fig. 2

which the viral suspension is composed of complex particulates of differing over-all sensitivity.

Relevance to Margin of Safety: These considerations have a practical bearing on the manufacturing problems, since heterogeneity that results in variation in the sensitivity of particulates to formaldehyde can operate to remove the so-called built-in factor of safety. This consequence, in a simplified example, is demonstrated in Figure 2.

Curve A describes the inactivation reaction proceeding at a commonly observed rate, the initial concentration of live virus being 1,000,000 particles per ml. (1.0×10^6). Curve B describes a similar reaction proceeding at a rate one-half as fast as Curve A, the initial live-virus concentration of these particles being 1000 per ml. Curve C describes a situation in which the rate of another set of particles is one-fourth of A, the initial live-virus concentration of this set being 32 particles per ml. The composite inactivation curve of a mixture of the three is given as Curve D. Starting with a suspension composed of the three types of particles in the assumed concentrations, the summation of the three reactions will be dominated by the reaction rate of A initially and by C at a later time.[5]

Viewed in this light, it may be concluded that observations on the initial phase of the reaction cannot be taken as the determining basis for predicting the remaining course of the reaction.

There are additional factors that might be operative and that might bear on the interrelated combination of inactivation and safety. The

suspending solution contains protein, coagulable by formaldehyde, which might favor the formation of viral particulates occluded in protein precipitates not generally distinguishable visually. Such particulates would be in a position of relative inaccessibility to formaldehyde.

The extent to which a combination of these factors influences the course of inactivation will also be reflected in the extent to which the total curve of actual inactivation departs from that of a first-order reaction. Unfortunately, the total curve is not measurable.

Analysis of Safety Testing: A safety test, generally speaking, is a laboratory procedure utilized as part of quality control of a product to separate that which is unsuitable from that which is suitable for the use intended. A major factor of safety, according to the original conception, was built into the process of inactivation. The safety tests were designed to demonstrate contamination resulting from accidents in manufacture as well as residual live virus, which the process was believed to reduce to an immeasurably low and harmless level. However, insofar as the process departs from the theoretical conception, whether because of deficiency in conception or because of inadequacies in the handling of the process, the testing becomes of increasing importance both as a guide to processing and as a final determinant of safety.

Two tests are used to determine the presence or absence of live virus in the quality control of the vaccine: the tissue-culture test and the monkey test.

The tissue-culture test had not been examined prior to April 1955 in a manner that permitted an estimation of its sensitivity. That is to say, it had not then been determined at what concentration of infective particles and under what circumstances a positive demonstration of live virus could be expected in a significant proportion of tests run. The psychology of last spring seems to have made this an unessential.

More recently, however, certain assumptions were made as a basis for analysis of the sensitivity and reliability of the tissue-culture test.[5] These will in general provide an estimate of reliability higher than actually obtains. The assumptions were as follows: 1) Residual live virus can be defined in terms of the number of discrete infective particulates contained in a unit volume of antigen suspension; 2) random distribution of these particulates obtains; 3) one infective particulate is an effective dose for a sensitive tissue-culture preparation; and 4) the

TABLE I.—SAMPLE SIZES REQUIRED AT SPECIFIED LEVELS OF CONCENTRATION, MOST SENSITIVE TEST

Probability of Negative Test	*Assumed Concentration of Infectious Units Per Liter*		
	5	*10*	*100*
.10	460 ml.	230 ml.	23 ml.
.05	600	300	30
.01	920	460	46
.001	1380	690	69

TABLE II.—SIZE OF SAMPLES USED PRIOR TO MAY 22, 1955

Laboratory	*Size of Sample (ml.)*	*Chances of Picking Up Positive Lot*
A	210	70%
B	1360	99
C	1600-2100	9.9+
D	325	80
E	180	65
F	1500	99.9+

volume of virus suspension is no less than ten liters.

With appropriate statistical theory, using the Poisson distribution, applicable in this case, certain probabilities were calculated. These are summarized in Table I, which shows the probability of failure to demonstrate the presence of live virus at different levels of concentration when different size samples are tested. If the upper level of acceptable viral concentration is set at five infective units per liter, the chances of picking up such a positive lot using the volumes commonly tested by each of the manufacturers prior to changes in the minimum requirements were as given in Table II.

It is apparent that different standards of acceptability as regards the possible presence of infective particles were inherent in the processes of the different manufacturers under the April 12 requirements. This

TABLE III.—PER CENT OF SINGLE STRAIN LOTS TESTING POSITIVE

Laboratory	*No. Lots*	*Per cent Positive*
All Labs.	812	11%
A	97	21
B	237	19
C	85	8
D	267	5
E	126	2

variation was possible under those requirements because of their provision that the minimum volume used in the test should be directly proportional (0.1 per cent) to the total volume of the pool under test. Established statistical principles indicate, however, that to ensure with a high degree of probability that the concentration of live-virus particles in any lot will be below a prescribed level, a sample of fixed minimum size must be tested, rather than one proportional to the size of the lot. The calculations assume that no lot contains less than ten liters.

MANUFACTURING EXPERIENCES

Let us turn now to the experience of industry to determine the extent to which these theoretical considerations were applicable. In manufacturing practice, the process of inactivation of the poliomyelitis virus was often characterized by a curvilinear rather than a straight-line relation between time and the log of the residual live-virus concentration in the initial phases of the reaction. This was also evidenced by the occurrence of positive tissue-culture tests from 9 to 12 days after the beginning of the inactivation process.

It was also apparent that the inactivation process had not been carried out in such a way as to yield consistent results in the hands of some members of the industry. That is to say, the initial test performed at the time when virus was expected to have been inactivated showed that an appreciable proportion of single-strain pools still contained live virus. The extent of this is summarized in Table III. This was an important finding. It demonstrated that the departure of the process

TABLE IV.—PER CENT OF POLYVALENT LOTS TESTING POSITIVE

Laboratory	*No. Lots*	*Per cent Positive*
All Labs.	127	13%
A	21	33
B	34	9
C	22	23
D	8	—
E	42	4

from one of systematic predictability greatly diminished the factor of safety believed to be inherent in the basic process. Consequently, principal reliance had to be placed upon the results of the tissue-culture tests for the exclusion of live virus in the final vaccine.

A second feature of the data may be seen by examining the safety tests performed on the trivalent pools immediately after combination. A summary of this is given in Table IV. It will be seen that live virus was frequently found in a trivalent pool when this had been presumed to be free of live virus on the basis of testing the monovalent pools that composed it.

It had to be concluded, therefore, that the tissue-culture test for active virus, as applied to the single-strain pool under the minimum requirements of April 12, was not an adequate process guide, nor did it provide sufficient assurance that live virus was not present in the trivalent pool in unacceptable concentrations. This emphasized the need to modify the tissue-culture tests for processing purposes and to fortify the final safety test performed on the trivalent pool before subdivision and packaging.

A third feature of the data, which can be seen more readily in the detailed rather than the summary tables, is an almost random distribution of positives in relation to the processing data of certain of the manufacturers. Positive tests had occurred in a sufficiently high proportion of cases to minimize the reliability of a single subsequent negative test, whether used for processing control purposes or as a final indication of safety of the trivalent pool.

Formulation of Amended Requirements (May 1955)

As a result of the considerations so far discussed, it has been possible to develop modifications of the requirements controlling the manufacture of poliomyelitis vaccine as these relate to safety.[6] A method that allows a concentration of live virus at no more than five tissue-culture infective units per liter once in a hundred thousand lots is believed to be a satisfactory objective and one within reach. Safety testing and processing have been revised to achieve this objective as follows.

Single strain pools are now inactivated with 1:4000 formaldehyde at 37°C for such a period of time as will permit two consecutive negative tissue-culture tests on samples separated by a three-day interval prior to the termination of inactivation. In order to ensure the exclusion of live-virus particulates above a minimal concentration, the volume of a single-strain pool used for each safety test must be not less than 500 ml.

The trivalent pool is to be considered suitable for vaccine manufacture if a negative tissue-culture test is obtained utilizing 1500 ml. of the pool in the tissue-culture test and employing two different tissue culture preparations.

As a final safeguard against accidental breaks in the over-all production routine, the manufacturer is required to test a random sample of vaccine from final containers of each lot for live poliomyelitis virus. The monkey safety test has been shifted from its previous position, where it was applied to the trivalent pool, and has now been adapted for use as the final-container test. At least five monkeys are inoculated with samplings of each filling lot, and there must be a minimum of 20 monkeys for each manufacturing lot from which these fillings are derived.

Tests made on these animals include tests for potential susceptibility, which requires the demonstration of the absence of circulating antibody in the serum prior to inoculation. In addition, samples of spinal cord segments are removed after 18 days for histologic study and to permit attempts to isolate virus in cases that are histologically questionable. An effort has been made to increase the sensitivity of the monkey test by increasing the susceptibility of the test animal, by increasing the number of monkeys used, and by injecting each animal with vaccine

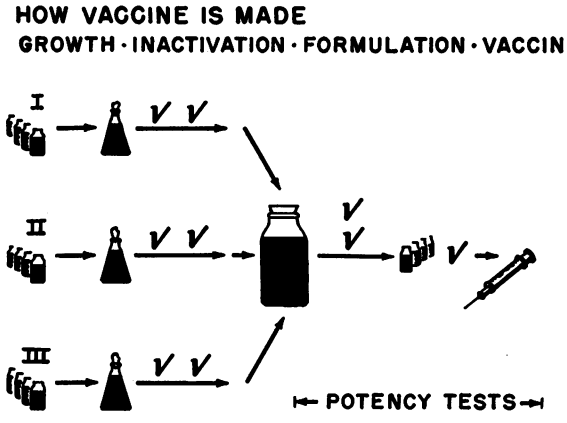

Fig. 3

not only intracerebrally, but also intraspinally and intramuscularly. To increase the susceptibility of the animal, the virus injections are accompanied by injection of 200 mg. of cortisone acetate into the left leg muscles and of 1 ml. of procaine penicillin into the right arm muscles.

As an additional safety test, conducted to establish the potency of vaccine administration via the route usually used in man, 12 monkeys are inoculated intramuscularly, each with 3 ml. of undiluted vaccine given in divided doses and at weekly intervals. These monkeys were formerly considered only as part of the potency test, but can now be considered as an integral part of the monkey safety-testing scheme. They receive an additional intracerebral injection and are studied histologically for evidence of lesions in the spinal cord.

The entire production and testing process is represented in Figure 3. Individual tests are indicated by checks, the final check in the process representing the monkey test. Potency tests are performed at any time after combination of the single-strain pools.

Application of the Revised Requirements: The application of the new requirements to the production of poliomyelitis vaccine was expected to result in a product essentially free of live virus. Negative results at the recommended test volumes in two consecutive tests during initial inactivation or following a single reinactivation period for each single-strain pool, and a third negative following the mixture of the

pools to form the trivalent pool, should ensure that the final vaccine contains five or more infective particulates per liter less than once in 100,000 times.

Additional operational refinements were believed possible in the routine production of the vaccine which should result in further improvements in product, lessening of costs, and no loss in safety. The contemplated refinements have been discussed with the manufacturers at length. These included the substitution of a less virulent Type-1 strain for the Mahoney strain. This should be in effect for next fall's vaccine. Other contemplated refinements are control tests to document sensitivity of tissue cultures, concentration of the viral suspension before applying the safety tests, the conduct of the safety tests themselves, and modification of physical arrangements during processing. Each of these requires further developmental research prior to application.

The application of the new minimal requirements met with outstanding success in one respect but not in another. It succeeded in placing a safe vaccine on the market, as judged by its use in more than seven million children. However, the inability of some of the manufacturers to produce in quantity resulted in a continuing shortage of vaccine in terms of the national need and demand.

On May 26, 1955, the Surgeon General appointed a Technical Committee on Poliomyelitis Vaccine. The responsibility of this Committee was twofold. First, it was to advise, on a lot-by-lot basis, on the release of vaccine for public use. Second, it was to establish and conduct with industry and our Division of Biologics Standards a collaborative study, developmental in nature, aimed at bringing into being at as rapid a rate as possible the several advances in vaccine production and testing that were envisioned in the initial investigation of the problem. The objective was not only an improved product, but the breaking of bottlenecks that impaired volume production. The bulk of the work has been performed by industry, with leadership in the collaborative effort being furnished by Technical Committee members. The details of this work will shortly find their way into the scientific literature.

Generalizing, one might say that studies completed or under way seem to have solved a number of problems of critical importance.[7]

As to safety, we believe the revised tissue-culture safety test has operated effectively, and that the new monkey test will enhance greatly the sensitivity of the procedure.

Abundant evidence is available to the effect that the particles of protected virus postulated in the June technical report do, in fact, exist. It is now known, however, that suitable filtrations placed at strategic points in the manufacturing process can obviate this difficulty.

The combination of process and safety improvement makes, we believe, the presence of the Mahoney strain in the current vaccine less critical. However, as still another added factor of safety, it can and will be replaced—but this will require more time to evaluate the many factors involved in a process as complex as this.

In summary, then, one might say that under the Technical Committee's leadership, industry has demonstrated, after a very trying period, that it can produce a vaccine effective in practice, and we are assured that an adequate amount can be produced under the present requirements.

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